10-26-2015

Paula Stoller
Valparaiso University
10026 Lincoln Court, Crown Point, IN 46307
(219) 577-5217
Paula.Stoller@valpo.edu

Effect of habitat and predator presence on blow fly oviposition in Michigan

PAULA R. STOLLER, Valparaiso University, 1610 Chapel Drive East, Valparaiso, IN 46383,

USA

KRISTI N. BUGAJSKI, Valparaiso University, 1610 Chapel Drive East, Valparaiso, IN 46383,

USA

ABSTRACT

Forensic entomology uses data derived from insects to aid in criminal investigations. Blow flies (Diptera: Calliphoridae) are usually the first insects to arrive at a crime scene and their quick appearance on carrion is the foundation for post mortem interval (PMI) estimations. Two areas that could potentially affect oviposition timing are the habitat carrion is in, and if blow fly predators are present. This study examined the effect of habitat (prairie vs forest) and the presence of predators (Hymenoptera: Vespidae) on blow fly oviposition timing. Research was conducted from June 1-August 10, 2015 at Pierce Cedar Creek Institute in Barry County, Michigan. Forest and prairie habitats were compared in observation trials that took place between 6-12 hours after sunrise and the timing of oviposition was noted. Manipulation studies were conducted to determine if odor or visual cues from predators are a stronger oviposition deterrent. Three bait cups contained only chicken liver and served as controls. Three bait cups had a pinned wasp placed on the chicken liver to serve as a visual manipulation. An odor manipulation consisted of crushing two wasps and sprinkling the crushed pieces over the chicken liver. No significant differences were found in oviposition timing or occurrence in the manipulation trials, or between prairie and forest sites. Temperature and humidity were not correlated with oviposition timing, which was surprising. *Lucilia coeruleiviridis* (Macquart) was the dominant species found, comprising about 90% of collected specimens. The data confirm previous reports that *Lucilia* species are first to arrive on carrion. These data provide evidence that the type of habitat a body is placed in should not affect PMI calculations.

KEYWORDS: blow flies, forensic entomology, habitat, oviposition, predation

Introduction:

Forensic entomology is the use of insects in the criminal justice system (Greenberg 1991; Haskell & Williams 2008; Byrd & Castner 2010). There are three main areas of forensic entomology: urban, stored product pests, and medico-legal (Catts & Goff 1992; Hall 1995; Byrd and Castner 2010.). Medico-legal forensic entomology focuses on the use of insects in determining the amount of time that has passed since insect colonization. Colonization by blow flies (Diptera: Calliphoridae) usually occurs within the first few hours after death and is used to estimate the postmortem interval (PMI) (Haskell & Williams 2008). The PMI is the period of time between death and corpse discovery. Establishing the PMI is important to investigators because it helps eliminate suspects, or validate testimonies.

A number of different factors can influence blow fly oviposition (egg laying) such as weather (Mann *et al.* 1990), temperature (Ames and Turner 2003), chemicals (Goff 1993), and potentially insect predators. In a previous experiment determining diurnal oviposition preferences, predatory wasps were observed on the bait cups on numerous occasions. The literature lacks information on the presence of predators role in blow fly oviposition and if sight or smell is a larger oviposition deterrent.

Archer and Elgar (2003) showed that olfaction is important in multiple ways for blow flies finding decomposing carcasses. Blow flies have the ability to distinguish chemical signatures for different stages of decay (Archer and Elgar 2003). Blow flies also depend on olfactory stimuli for attraction and orientation and blow flies can sense a host animal as far as 10 m upwind (Wall and Fisher 2001).

It is possible that this keen sense of smell that is used to detect hosts might also be used to sense predators and avoid ovipositing when predators are present. Archer and Elgar (2003)

studied the predation of wasps and ants on blow flies. The "larger" blow flies (*Calliphora* species) tended to escape predation more often than the "smaller" (*Lucilia* species) blow flies. Wasps and ants only attacked during the first four days of decomposition. Blow flies that came after the first four days of decomposition did not have any risk of predation (Archer and Elgar 2003).

There is an absence of published data on blow fly habitat preferences in the United States, and this research provides data on the first blow flies to arrive in forest and prairie habitats in Michigan. A study done in England by Cruickshank and Wall (2002) found that *Lucilia* species were caught in warmer, more humid field sites. They also found that in the absence of odors, *Lucilia sericata* (Meigen) aggregated near the hedgerow at the edges of farm fields. Vanin *et al.* (2008) studied *L. sericata* in northern Italy and found that the species does not show a habitat preference in rural regions with urban sprawl.

Methods:

Research was conducted in summer 2015 from June 1 to August 10 at Pierce Cedar Creek Institute in Barry County, Michigan. Pierce Cedar Creek Institute is a 298 ha nature preserve that has forest, prairie, and wetland habitats as well as a small lake and two creeks running through the property. Table 1 outlines the number of replicates and sites used in all trials. Bait cups were used to attract blow flies for oviposition and consisted of a clear plastic cup with ¼ inch of vermiculite in the bottom and a foil cup with approximately 60 grams of chicken liver placed inside. The chicken liver was aged in a fume hood overnight (approximately 14 hours) and was placed into foil cups in the morning. The covered cups were placed on the ground at the sites 4 hours after sunrise. Six hours after sunrise the lids were removed from the cups and observations began. Every half hour the cups were checked for blow fly eggs, flesh fly larva, and

the presence of blow flies and other insects. Once oviposition was observed, the cup was covered, labeled, and removed from the field. Observations ended 12 hours after sunrise. Bait cups with blow fly eggs or flesh fly larva were placed in the fume hood and reared to the third larval instar stage and identified to species for blow flies and family for flesh flies (Stojanovich et al. 1962; Whitworth 2008). Flesh flies were identified to family due to the difficulty in species identification of this group.

The manipulation studies, which were separate from observation studies, utilized the forest habitat at Pierce Cedar Creek Institute. Nine bait cups, as described above, were used for each trial, and the experiment was repeated ten times. Three bait cups only contained chicken liver and served as controls. The first manipulation was visual, and three bait cups had a pinned wasp (Hymenoptera:Vespidae, *Parancistrocerus leionotus* (Viereck)) placed on the chicken liver to serve as the visual manipulation. The odor manipulation consisted of crushing two wasps (Hymenoptera:Vespidae, *P. leionotus*) and sprinkling the crushed pieces over the chicken liver. Bait cups were covered and randomly placed into stands (using a random number generator) in the fields at 4 hours after sunrise. The stands consisted of a yellow wooden stake with a yellow platform attached to it. Observations followed the same protocol as described for observation studies.

Ambient weather data were collected from a weather station located at Pierce Cedar Creek Institute. Temperature and humidity were recorded every hour during the field season.

Analysis:

Data were analyzed using SPSS statistical software (SPSS 2009). A one way ANOVA examined differences in the frequency of oviposition and timing of oviposition events for manipulation trials, as well as manipulation and observation comparisons. Independent sample t-tests were

used to determine significant differences in the number and timing of oviposition events for prairie and forest observation trials. Correlations examined the relationship between temperature and humidity, and the timing of oviposition.

Results:

No significant difference existed in the frequency of oviposition in manipulation treatments (F=1.184, df=98, p=0.311). The timing of oviposition for treatments in manipulation trials was also not significant (F=0.270, df=72, p=0.764). When all prairie and forest sites were combined, there was no significant difference in oviposition timing between habitats (t=-0.196, df=84, p=0.845). Three different trails were tested independently and all results showed no significant difference in oviposition timing (red trail: t=0.389, df=24, p=0.701; purple trail: t=-0.143, df=28, p=0.888; yellow trail: t=-0.736, df=28, p=0.468).

When observation and manipulation studies were compared, a significant difference was found (F=4.705, df=153, p=0.001). Forest observations were significantly different than control manipulation (p=0.024), and pinned manipulation (p=0.040). Prairie observations were significantly different from control manipulation (p=0.022), and pinned manipulation (p= 0.036).

Manipulation trials had oviposition between 6-12 hours after sunrise, which was 0-6 hours after exposure for oviposition. Average oviposition times can be found in Figure 1.

Abiotic factors were examined for significant relationships to oviposition timing. Linear regression analysis showed no correlation between temperature and time of oviposition (r^2=0.012) (Fig. 2) or humidity and oviposition time (r^2=0.057) (Fig. 3).

A total of 10,043 maggots were identified from 9 observation and 10 manipulation trials. *Lucilia coeruleiviridis* (Macquart) was the dominant species found, comprising around 90% of all collected specimens. Species composition differed between manipulations (Fig. 4), and

between prairie and forest trails (Fig. 5). In manipulation trials controls were comprised of 92% *L. coeruleiviridis*, 0.2% *Lucilia sericata* (Meigen) and 7.8% Sarcophagidae species. Cups containing crushed wasps had 90% *L. coeruleiviridis*, 1% *Phormia regina* (Meigen) and 9% Sarcophagidae species. Pinned manipulations were the least diverse, comprised of 89% *L. coeruleiviridis* and 11% Sarcophagidae species (Fig. 4).

The yellow trail prairie and forest were the least diverse of the observation trials, both comprised of 100% *L. coeruleiviridis*. The red forest trail was comprised entirely of blow fly species, it had 90% *L. coeruleiviridis* and 10% *L. sericata*, while the red prairie had 97% *L. coeruleiviridis*, 0.8% *P. regina*, and 8.2% Sarcophagidae species. The purple forest trail had 97% *L. coeruleiviridis* and 3% Sarcophagidae species while the purple prairie trail had more Sarcophagidae species, with 91% *L. coeruleiviridis* and 9% Sarcophagidae species (Fig. 5).

Discussion:

This study had results that were consistent with previous findings by Gruner *et al.* (2007) in Florida. Gruner *et al.* (2007) found *L. coeruleiviridis* to be the most abundant calliphorid species collected, comprising about 90% of the species collected on days 1-2 of the study in the summer. They reported that it was always the first to arrive at the fresh carrion and the first to deposit eggs (Gruner *et al.* 2007). This is similar to results found at Pierce Cedar Creek Institute, where *L. coeruleiviridis* represented about 90% of species collected (Fig 4, 5), and were the first blow flies recorded ovipositing.

Lucilia species are known to be early arrivers at carrion and therefore important in forensic entomology investigations (Byrd and Castner 2010). However, they are not always the dominant species found in succession studies. Previous work by Haskell (1989) was done in Northwest Indiana, which has a similar climate and is geographically close to Pierce Cedar

Creek Institute. He found that in the summer *P. regina* comprised 85% of the total specimens with the *Lucilia* species represented at 10% and *Cochliomyia macellaria* (Fabricius) 5% in Northwest Indiana. This is vastly different from the data found in this study, where *Lucilia* species dominated.

No significant differences existed in the timing of oviposition between prairie and forest sites, or between the manipulation trials. The timing of oviposition in manipulation studies was significantly later than observation studies (Fig 1). This could be due to the bait cups being placed into yellow stands in attempts to attract Hymenoptera (Chittka and Raine, 2006; Chittka and Menzel, 1992). In the observation studies, the bait cups were placed directly onto the ground, mimicking cadaver placement. If a body was elevated off the ground, it will potentially delay oviposition. This should be considered when estimating the postmortem interval.

In addition to timing, there were differences in blow fly behavior noted when there were living Vespidae (predatory wasp) present on the bait cups. Live wasps were usually observed in bait cups containing pinned wasps. With the pinned and crushed wasp manipulations, the blow flies tended to land on the stand or on the edge of the bait cup first before entering the cup and ovipositing. When there was a live wasp, or in some instances multiple wasps, present in the bait cups, the blow flies did not approach the cups until the wasp(s) had left the cup, and would then wait before ovipositing. If blow flies were in the cup and a wasp came back, they would fly away almost immediately. Blow fly oviposition in cups with live wasps almost always occurred after the wasp(s) had left. The pinned wasps did not affect blow fly oviposition, suggesting that the blow flies are not intimidated by the mere sight of the wasps. Crushed wasp also did not significantly delay oviposition. These results suggest that it is not independent visual or olfactory cues that warn blow flies of potential predators, but perhaps a combination.

Researchers thought that oviposition timing would be tied to ambient weather conditions, since there appeared to be a shift towards earlier oviposition in the warmer month of July. In June oviposition tended to occur later in the day, specifically between 7.5 and 12 hours post sunrise. In July, oviposition occurred between 6 and 11.5 hours after sunrise but moved to between 6 and 9.5 hours after sunrise at the end of July. When the temperature and humidity at the time of oviposition were plotted against oviposition timing, there were no significant correlations (Fig 2, 3). This is surprising, because blow flies are more active at warmer temperatures, so researchers assumed oviposition would occur earlier when the temperature was higher.

This research provides important information on blow fly behavior and oviposition timing to the field of forensic entomology. Blow flies changed their behavior in response to live wasps, but not crushed or pinned wasps. On average, oviposition was seen 7.5 hours after sunrise, and blow flies increased their activity as the day progressed. Oviposition timing is critical to post mortem interval estimations, and this study found that oviposition was not correlated with temperature or humidity, but to the time of day. Future research should continue to examine the timing of blow fly oviposition.

There are multiple different studies that could be done in the future to expand upon this research. One could be a behavior study examining live wasp and blow fly interactions. Interactions between the blow flies and live wasps were observed to be different than with pinned and crushed wasps so live waps might have an impact on oviposition. Another future project could involve leaving the bait cups out for longer periods of time to see if other species of blow flies will lay more eggs, since *Lucilia* are usually the first to arrive at carrion. A different future study could look into whether there is difference in oviposition between bait cups placed

on the ground or in stands. This would help determine if the stands used in this experiment had an impact on the time of oviposition.

Acknowledgements:

This project was funded through the Pierce Cedar Creek Institute's Undergraduate Research Grants for the Environment (URGE) program. We thank the Pierce family's generosity in their support of this program. Sara Syswerda and the staff at Pierce Cedar Creek Institute deserve thanks for their support during the field trials. Hannah Tomten assisted with field work. The College of Arts and Sciences at Valparaiso University also provided supplies for this project.

Literature Cited:

Amendt, J., Campobasso, C. P., Gaudry, E., Hall, M. J. R., & LeBlanc, H. N., Reiter, C. 2007. Best practice in forensic entomology—standards and guidelines. Int J Legal Med. 121: 90–104.

Ames, C., & Turner, B. 2003. Low temperature episodes in development of blowflies: implications for postmortem interval estimation. Medical and veterinary entomology, 17(2), 178-186.

Archer, M. & M. Elgar. 2003. Effects of decomposition on carcass attendance in a guild of carrion-breeding flies. Journal of Medical and Veterinary Entomology 17:263-271.

Byrd, J. & J. Castner. 2010. Forensic Entomology: The Utility of Arthropods in Legal Investigations, 2nd ed. CRC Press, Inc., Boca Raton, Florida. 681 pages.

Catts, E. & M. Goff. 1992. Forensic entomology in criminal investigations. Annual Review of Entomology 37:253-272.

Chittka, L., & Menzel, R. 1992. The evolutionary adaptation of flower colours and the insect pollinators' colour vision. Journal of Comparative Physiology. 171(2):171-181.

Chittka, L., & Raine, N. E. 2006. Recognition of flowers by pollinators. Current opinion in plant biology. 9(4):428-435

Cruickshank, I., & Wall, R. 2002. Aggregation and habitat use by Lucilia blowflies (Diptera: Calliphoridae) in pasture. Bulletin of entomological research, 92(02), 153-158.

Goff, M. L. 1993. Estimation of postmortem interval using arthropod development and successional patterns. Forensic Science Review, 5(2), 81-94.

Greenberg, B. 1991. Flies as forensic indicators. Journal of Medical Entomology, 28(5), 565-577.

Gruner, S. V., Slone, D. H., & Capinera, J. L. 2007. Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. Journal of medical entomology, 44(3), 509-515.

Hall, M. 1995. Trapping the flies that cause myiasis: their responses to host-stimuli. Annals of Tropical Medicine and Parasitology 89:333-357.

Haskell, N.H. 1989. Calliphoridae of pig carrion in northwest Indiana: A seasonal comparative study. M.S. thesis, Purdue University, West Lafayette.

Haskell, N. & R. Williams. 2008. Entomology and Death: A Procedural Guide, 2nd ed. Forensic Entomology Partners, Clemson, South Carolina. 182 pages.

Mann, R.W., Bass, W.M. & Meadows, L. 1990. Time since death and decomposition of the human body: Variables and observations in case and experimental field studies. Journal of Forensic Sciences 35: 103-111.

Shaumar, N. F., El-Agoze, M. M., & Mohammed, S. K. 1990. Parasites and Predators Associtated with Blow Flies and Flesh Flies in Cairo Region. Journal of the Egyptian Society of Parasitology 30:123-132.

SPSS for Windows, Rel. 18.0.0. 2009. SPSS Inc., Chicago, Illinois.

Stojanovich, C., H.D. Pratt & E.E. Bennington. 1962. Fly larvae: Key to some species of public health importance. US Department of Health, Education and Welfare. Public Health Service, communicable Disease Center, Training Branch, Atlanta, Georgia.

Voss, S. C., Spafford, H., & Dadour, I. R., 2009. Hymenopteran Parasitoids of Forensic Importance: Host Association, Seasonality, and Prevalence of Parasitoids of Carrion Flies in Western Australia. Journal of Medical Entomology 46(5):1210-1219.

Vanin, Stefano, P. Tasinato, G. Ducolin, C. Terranova, Silvano Zancaner, M. Montisci, S. D. Ferrara, and M. Turchetto. 2008. Use of *Lucilia* species for forensic investigations in Southern Europe. Forensic science international 177:1, 37-41.

Wall, R. & P. Fisher. 2001. Visual and olfactory cue interaction in resource-location by the blowfly, Lucilia sericata. Journal of Physiological Entomology 26:212-218.

Whitworth, T.L. 2006. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proceedings of the Entomological Society of Washington. 108(3):689-725.

Table 1. Experimental design for observation and manipulation trials

	Manipulation Trials	**Observation Trials**
Purple Trail	10 total trials throughout the summer. Each trial had 3 control cups, 3 crushed wasp cups, 3 pinned wasp cups.	3 trials total throughout the summer. Each trial had 5 cups in prairie, 5 cups in forest.
Red Trail	n/a	3 trials total throughout the summer. Each trial had 5 cups in prairie, 5 cups in forest.
Yellow Trail	n/a	3 trials total throughout the summer. Each trial had 5 cups in prairie, 5 cups in forest.

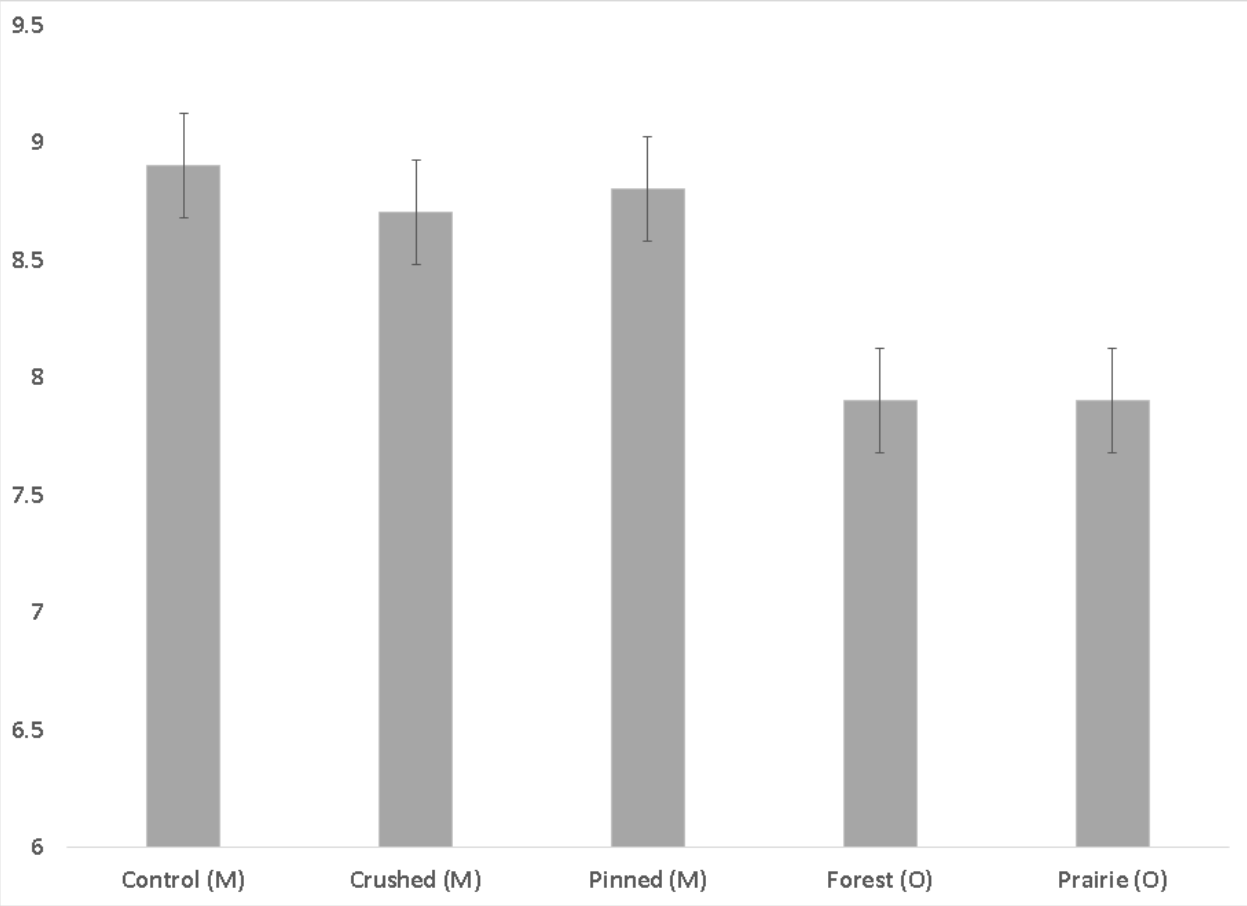

Figure 1. Average time of oviposition in hours after sunrise for manipulation (M) and observation (O) trials. A total of 10 manipulation and 9 paired observation trials were conducted. No significant differences were found in timing of oviposition for manipulation (F=0.270, df=72, *p*=0.764), or observation (F=0.270, df=72, *p*=0.764) trials. A significant difference (F=4.705, df=153, p=0.001) was found when all trials were compared.

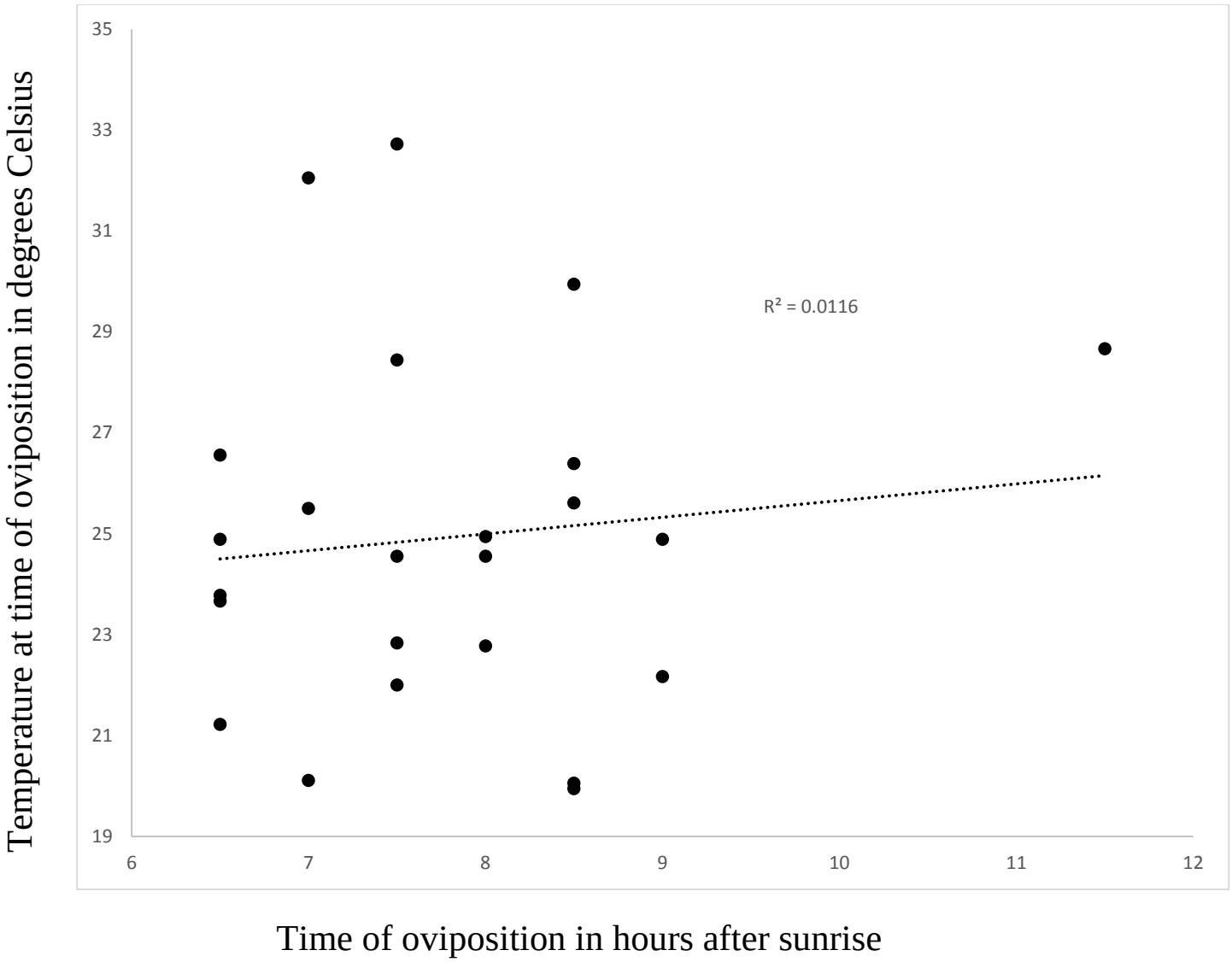

Figure 2. Ambient temperature at the time of oviposition is not correlated with the timing of oviposition in hours after sunrise (r^2=0.012, p=0.617).

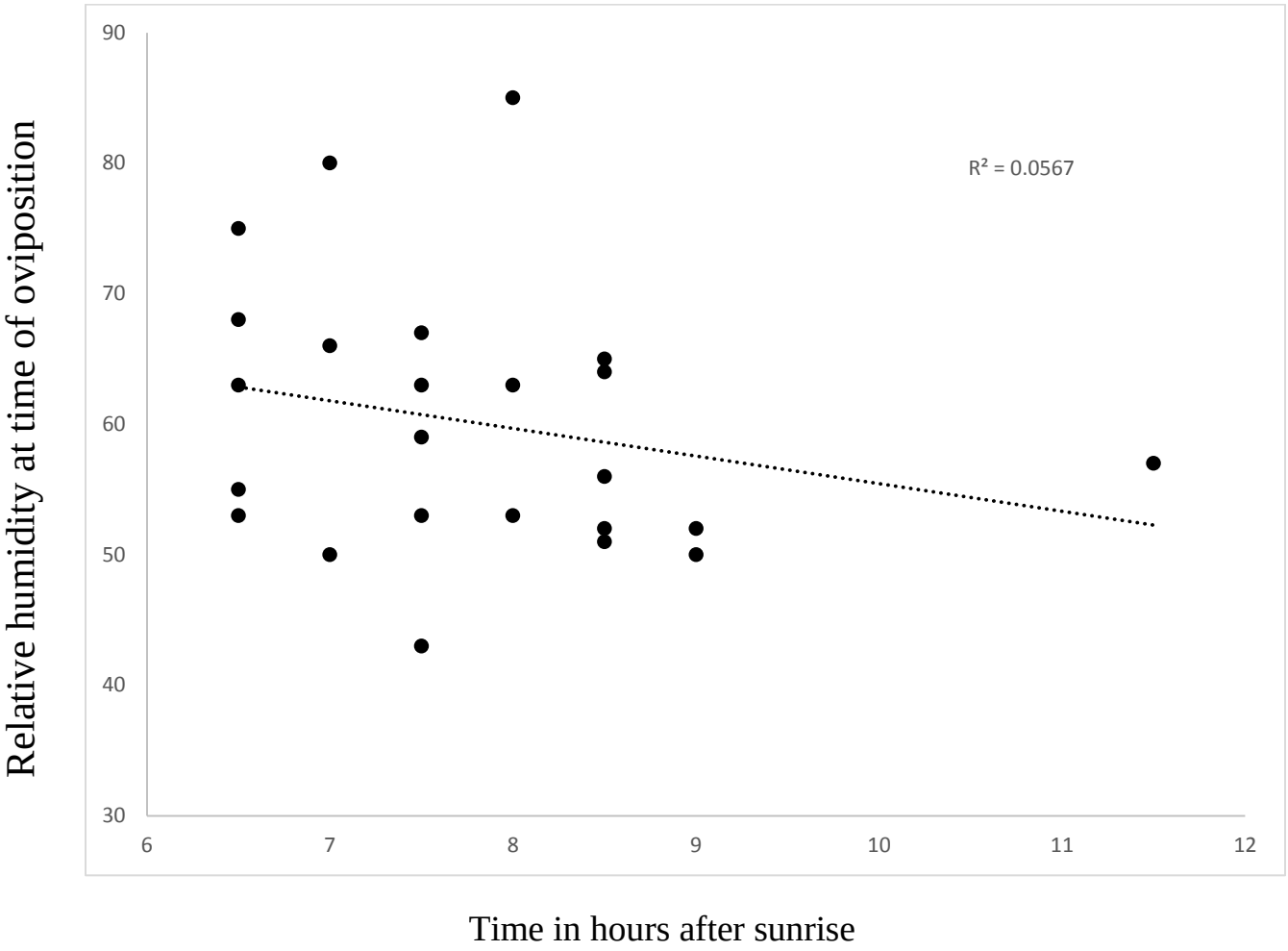

Figure 3. Humidity at the time of oviposition is not correlated with the timing of oviposition in hours after sunrise (r^2=0.057, p=0.262).

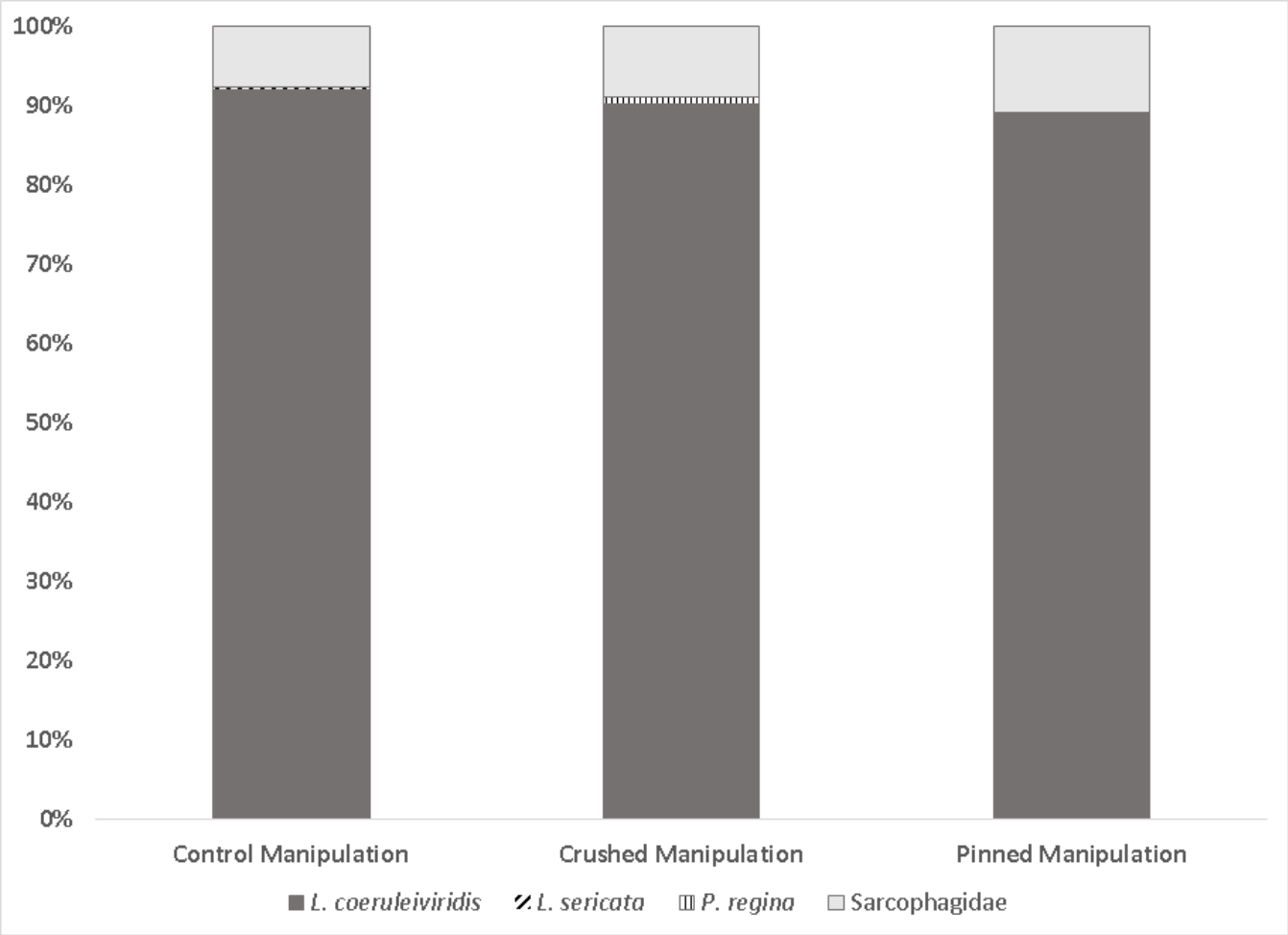

Figure 4. Species composition for control, pinned and crushed manipulation trials. A total of 3,038 larva were identified from 10 manipulation trials.

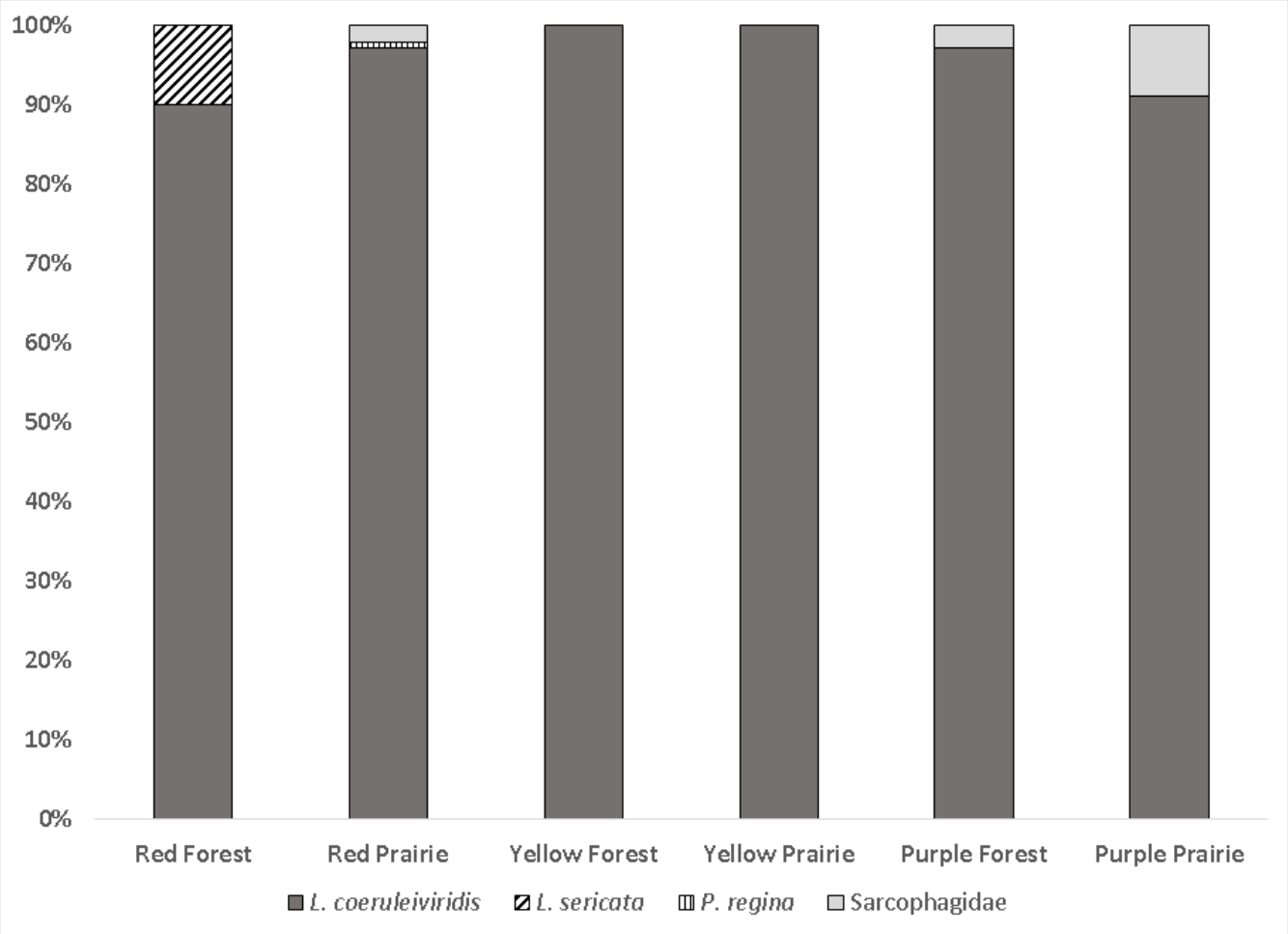

Figure 5. Species composition for the red, yellow and purple trail forest and prairie sites. A total of 7,005 larva were identified from 9 paired observation trials.